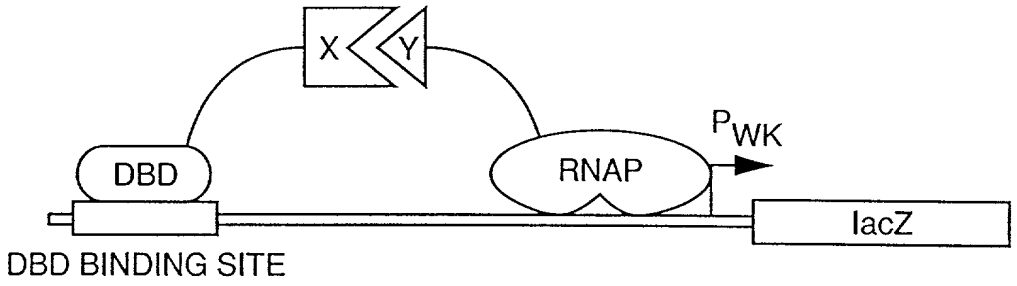


FIG. 1A

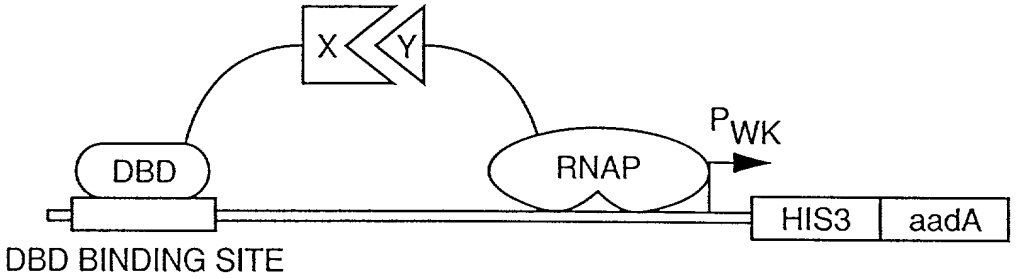


FIG. 1B

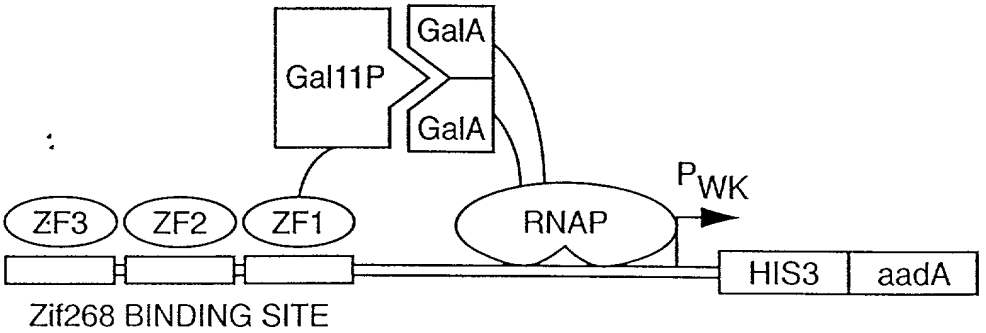


FIG. 1C

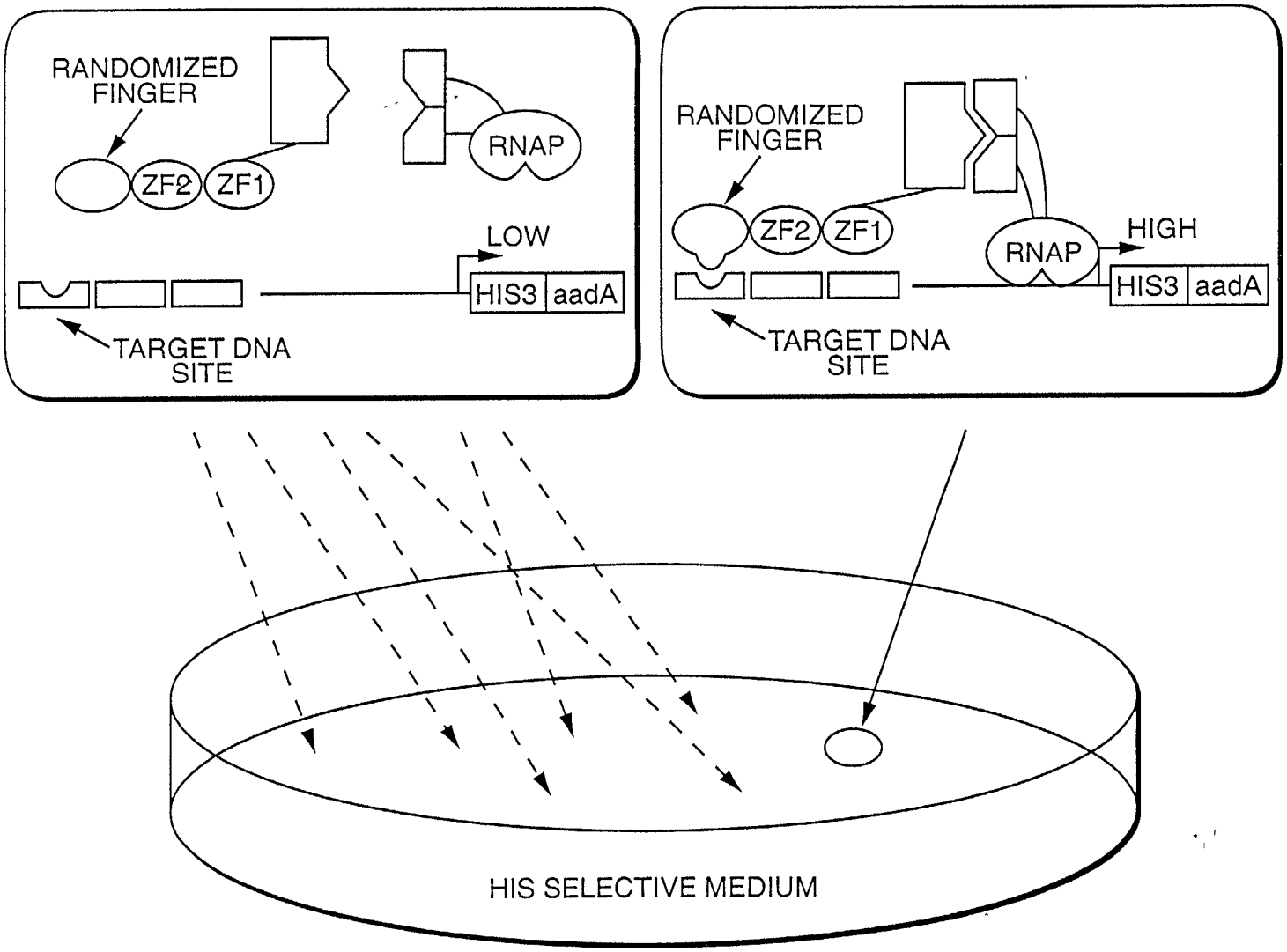

RANDOMIZED FINGER
ZF2
ZF1
RNAP
LOW
HIS3
aadA
TARGET DNA SITE
RANDOMIZED FINGER
ZF2
ZF1
RNAP
HIGH
HIS3
aadA
TARGET DNA SITE
HIS SELECTIVE MEDIUM

FIG. 2

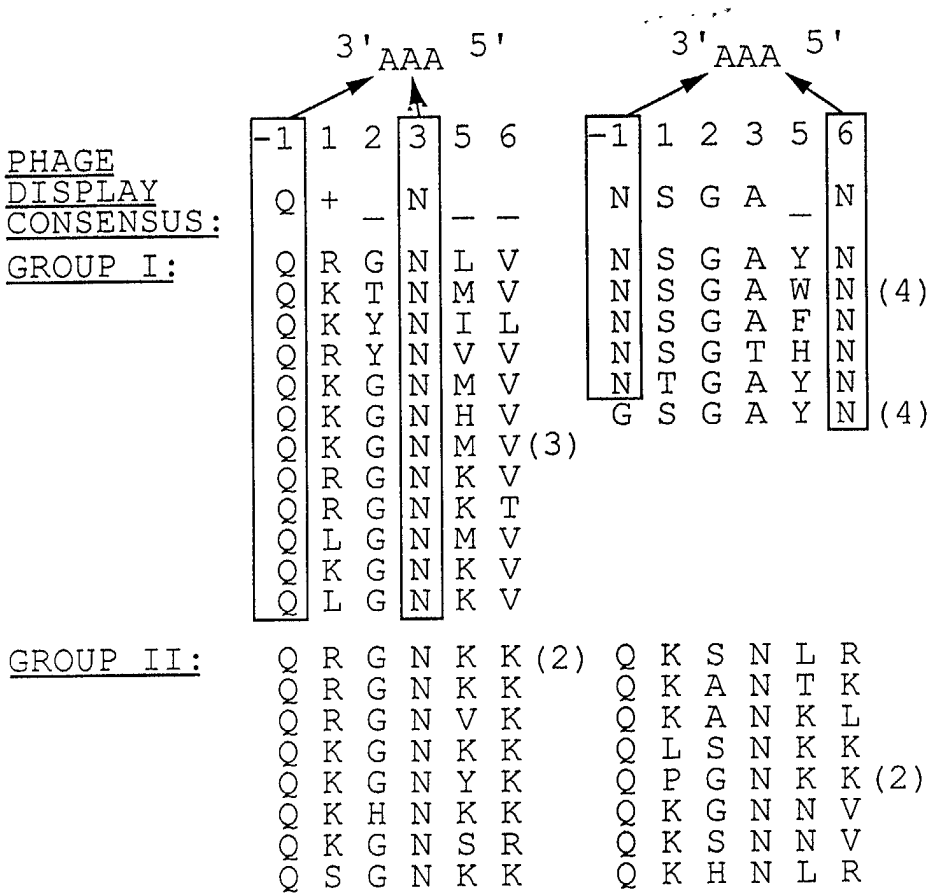

3' AAA 5'
3' AAA 5'
PHAGE DISPLAY CONSENSUS:
GROUP I:
GROUP II:
-1 1 2 3 5 6
Q + _ N _ _
Q R G N L V
Q K T N M V
Q K Y N I L
Q R Y N V V
Q K G N M V
Q K G N H V
Q K G N M V(3)
Q R G N K V
Q R G N K T
Q L G N M V
Q K G N K V
Q L G N K V
-1 1 2 3 5 6
N S G A _ N
N S G A Y N
N S G A W N (4)
N S G A F N
N S G T H N
N T G A Y N
G S G A Y N (4)
Q R G N K K(2)
Q R G N K K
Q R G N V K
Q K G N K K
Q K G N Y K
Q K H N K K
Q K G N S R
Q S G N K K
Q K S N L R
Q K A N T K
Q K A N K L
Q L S N K K
Q P G N K K(2)
Q K G N N V
Q K S N N V
Q K H N L R

FIG. 3A

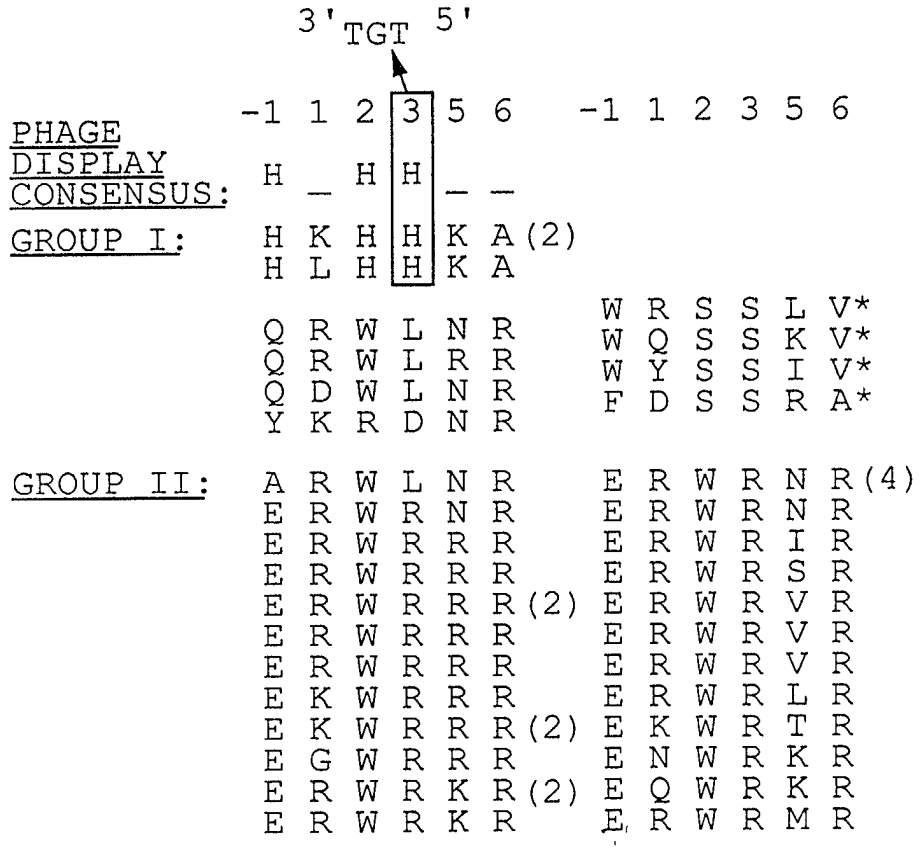

3' TGT 5'
PHAGE DISPLAY CONSENSUS:
GROUP I:
GROUP II:
-1 1 2 3 5 6
H _ H H _ _
H K H H K A(2)
H L H H K A
Q R W L N R
Q R W L R R
Q D W L N R
Y K R D N R
A R W L N R
E R W R N R
E R W R R R
E R W R R R
E R W R R R(2)
E R W R R R
E R W R R R
E K W R R R
E K W R R R(2)
E G W R R R
E R W R K R(2)
E R W R K R
-1 1 2 3 5 6
W R S S L V*
W Q S S K V*
W Y S S I V*
F D S S R A*
E R W R N R(4)
E R W R N R
E R W R I R
E R W R S R
E R W R V R
E R W R V R
E R W R V R
E R W R L R
E K W R T R
E N W R K R
E Q W R K R
E R W R M R

FIG. 3B

3' ACT 5'

	-1	1	2	3	5	6	
GROUP I: FIRST SELECTION:	N	S	G	S	W	K	
SECOND SELECTION:	N	S	G	S	W	K	(15)
	N	H	G	S	W	K	(2)
	N	S	G	S	H	K	
PHAGE DISPLAY CONSENSUS:	T	_	T	N	_	S	

FIG. 3C

	-1	1	2	3	5	6	-1	1	2	3	5	6	
FROM THE TATA SELECTION:	R	R	W	L	K	L	R	K	W	L	Q	L	
	R	K	W	L	K	L	R	K	W	L	R	L	(2)
FROM THE p53 SELECTION:	R	K	W	L	R	L	R	K	W	L	M	L	
	R	K	W	L	R	L	R	K	W	I	N	L	
	R	K	W	L	R	L	R	K	W	Y	Q	L	
	R	K	W	L	R	L							
FROM THE NRE SELECTION:	R	K	W	L	R	L	R	A	W	L	K	L	
	R	K	W	L	K	L	K	K	W	Y	R	L	
	R	K	W	L	K	L							

FIG. 3D

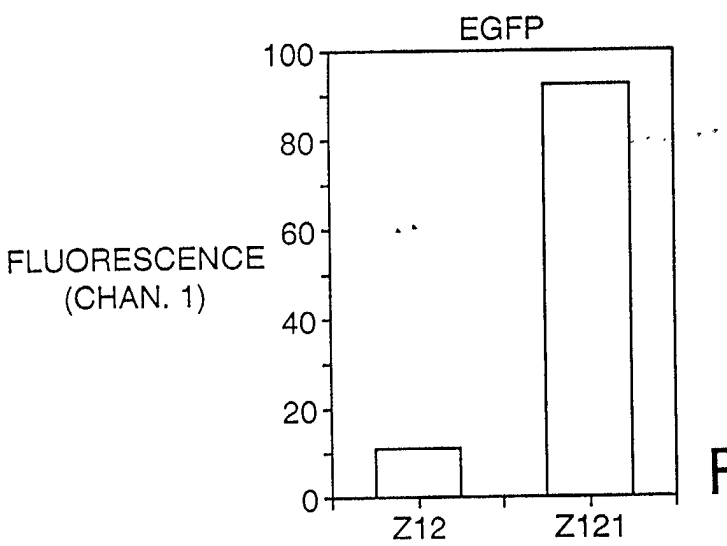


FIG. 4A

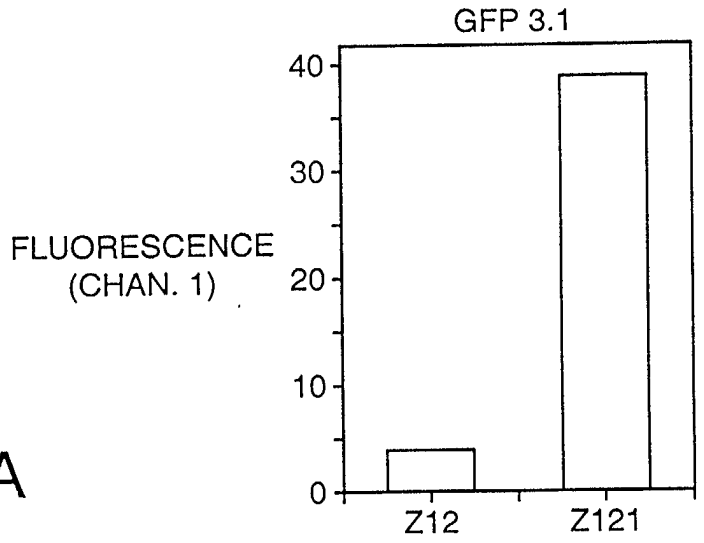


FIG. 4B

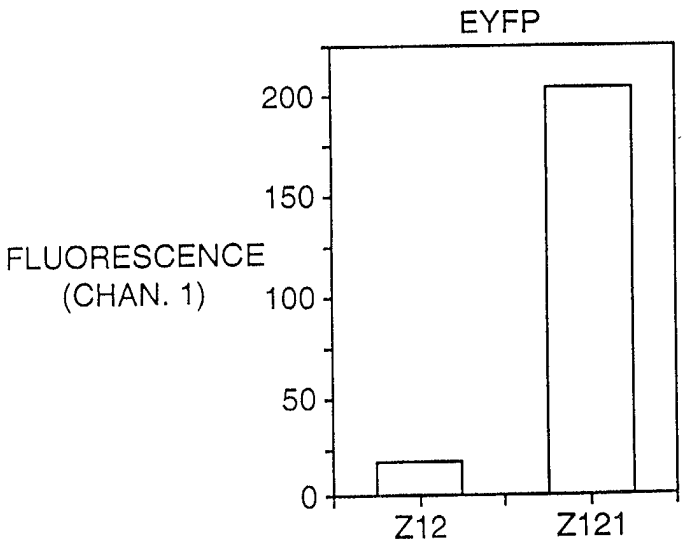


FIG. 4C

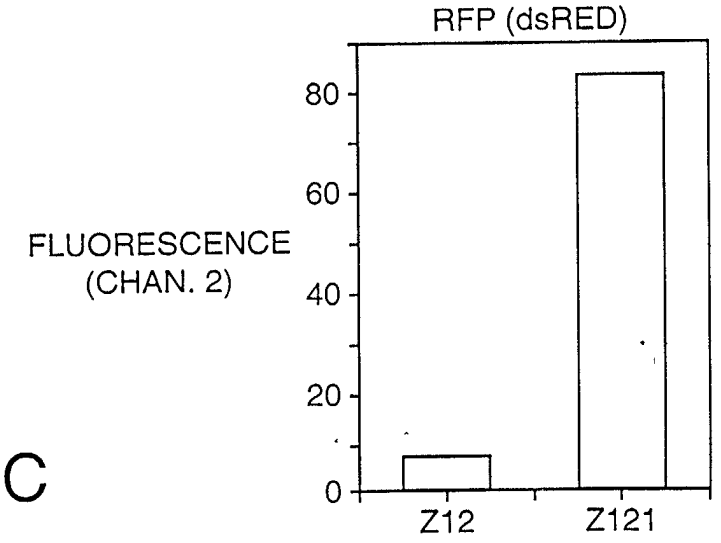


FIG. 4D

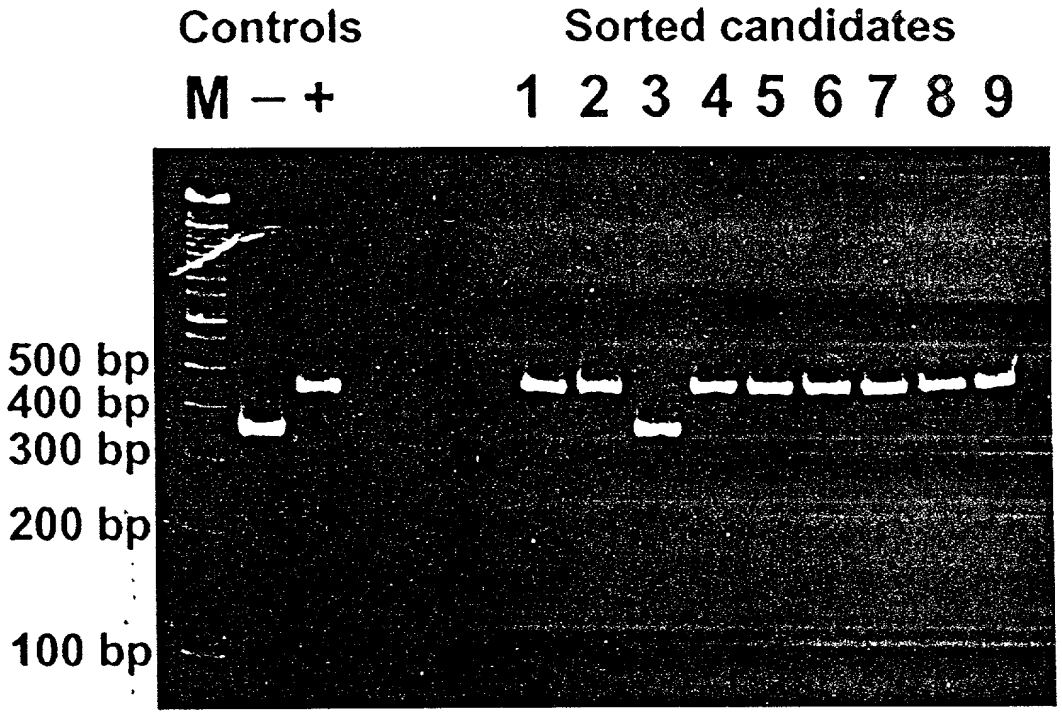

Controls
Sorted candidates
M – +
1 2 3 4 5 6 7 8 9
500 bp
400 bp
300 bp
200 bp
100 bp

Fig. 5

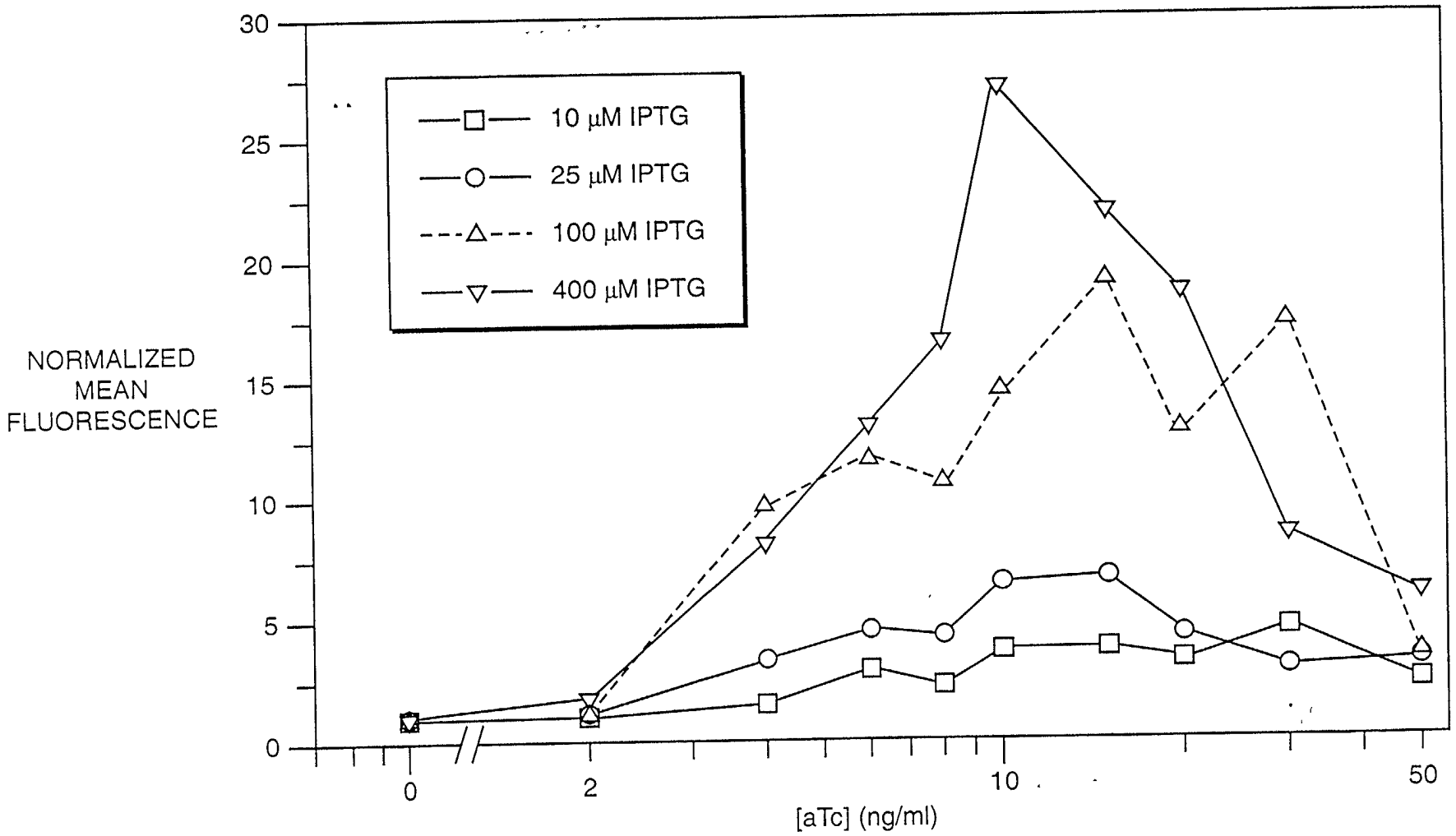

30
25
20
15
10
5
0
NORMALIZED MEAN FLUORESCENCE
10 μM IPTG
25 μM IPTG
100 μM IPTG
400 μM IPTG
0
2
10
50
[aTc] (ng/ml)

FIG. 6

REPORTER CONSTRUCT #2

REPORTER CONSTRUCT #1

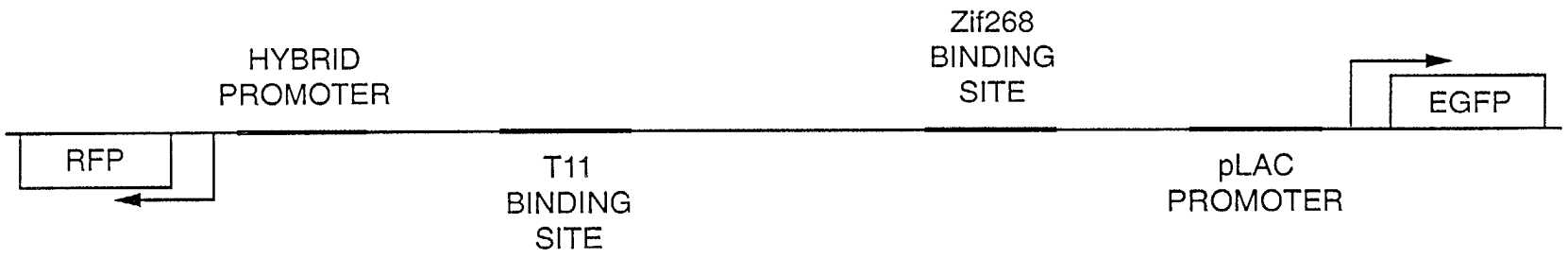


FIG. 7

TWO-COLOR REPORTER + Gal11P-T11 PROTEIN

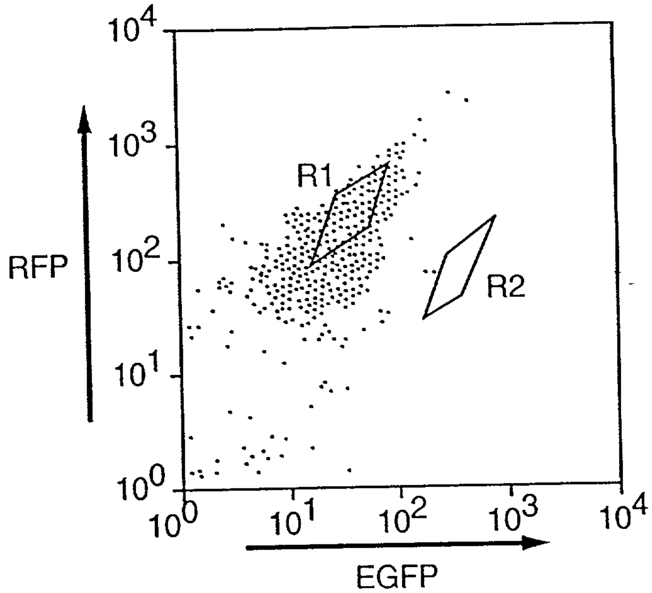


FIG. 8A

TWO-COLOR REPORTER + Gal11P-Zif268 PROTEIN

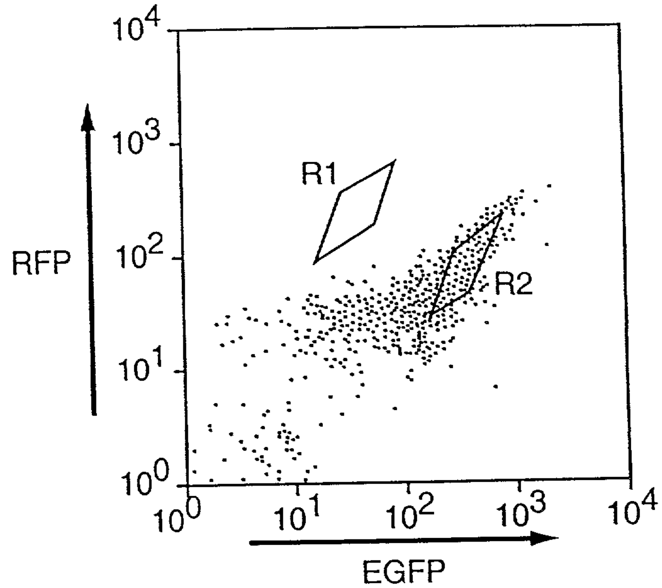


FIG. 8B

TWO-COLOR REPORTER + Gal11P-Z12 PROTEIN

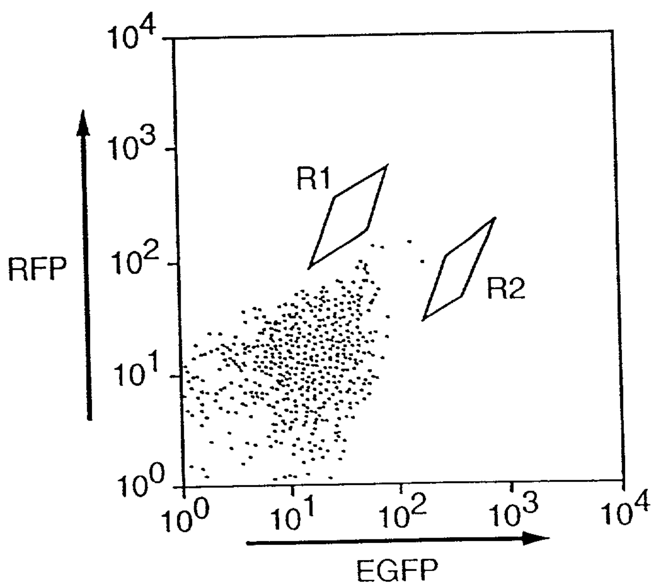


FIG. 8C

P53^{ZF} *IN VITRO* SITE SELECTION CONSENSUS SEQUENCE:

CXGGACACGTX

(WHERE X = NO CLEAR PREFERENCE)

IN VIVO SITE SELECTION LIBRARY

CGGGANNNNNG

(WHERE N = A MIXTURE OF A, G, C, AND T)

SELECTED CLONES:

SEQUENCE	# OF CLONES
CGGGACACGTG	9
CGGGACATGTG	5
CGGGACACGGG	2

SEQUENCE	FOLD ACTIVATION
CGGGACACGTG	18.6 ± 2.7
CGGGACATGTG	12.0 ± 0.5
CGGGACACGGG	12.6 ± 1.9

FIG. 9

FIG.10

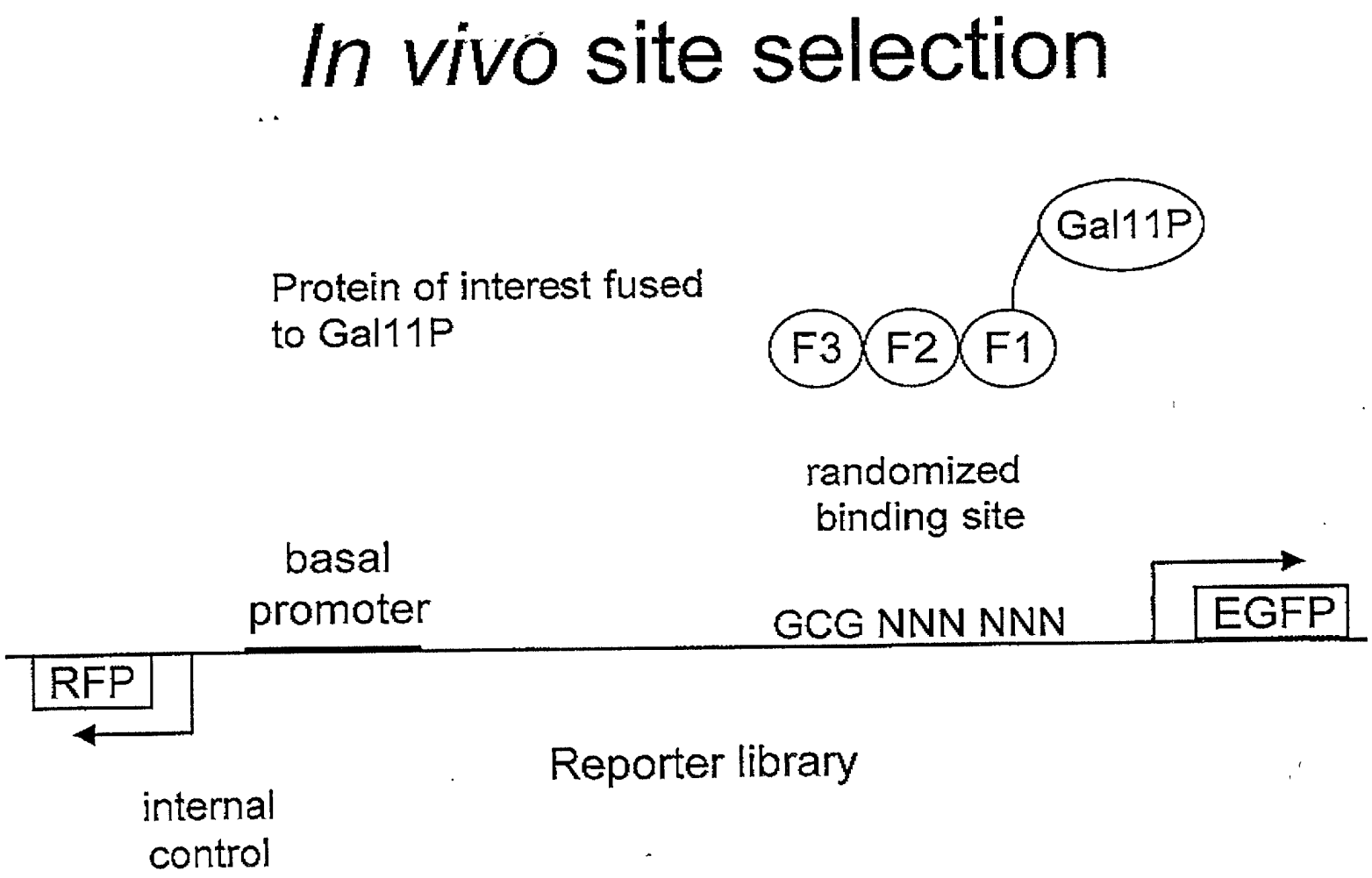

In vivo site selection
Gal11P
Protein of interest fused to Gal11P
F3
F2
F1
randomized binding site
basal promoter
GCG NNN NNN
EGFP
RFP
Reporter library
internal control

FIG. 11

Selecting Dimerization Domains

Tail to Tail

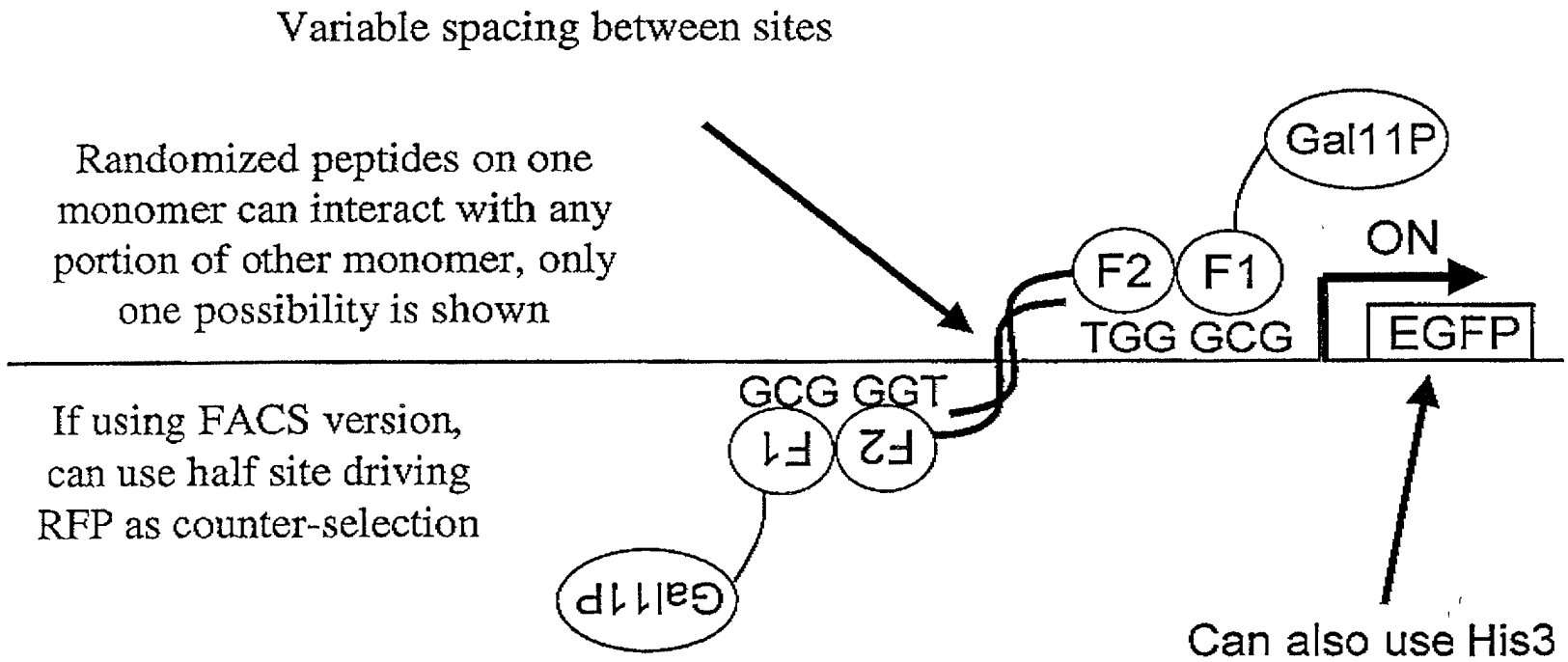
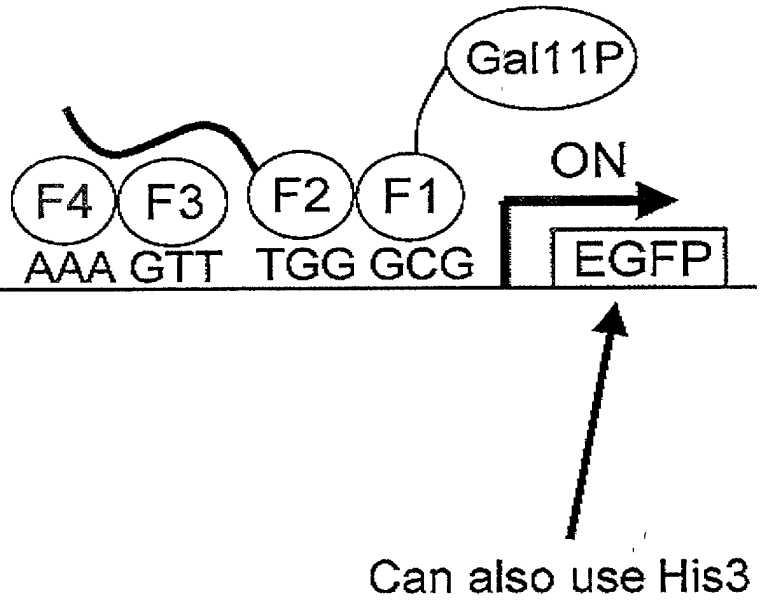


FIG. 12

Selecting Dimerization Domains

Head to Tail



If using FACS version, can use half site driving RFP as counter-selection

Can also use His3

FIG. 13

Selecting sequence-specific domains from random peptides

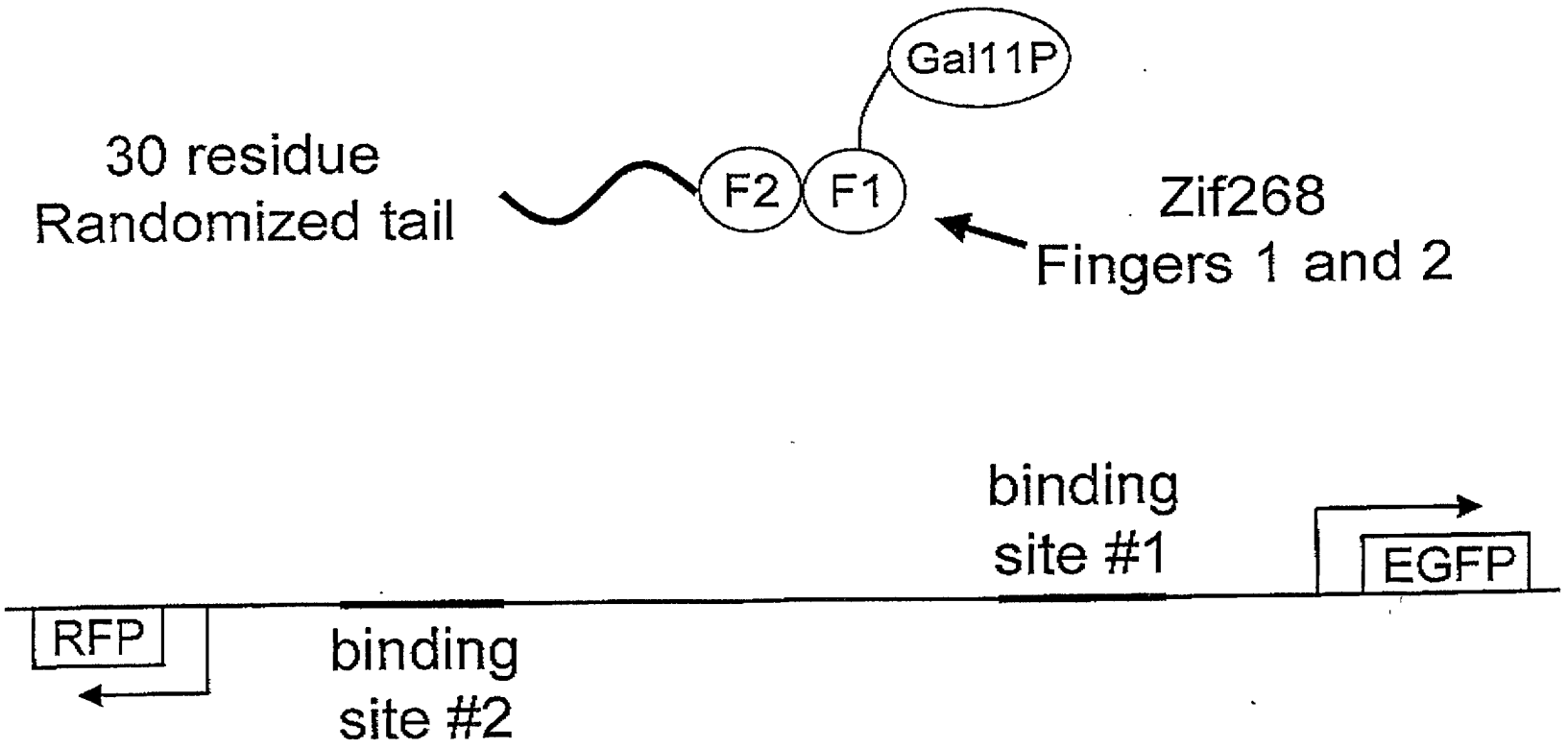
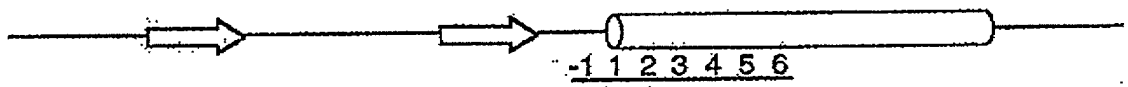


FIG. 14

Sequence of library and reporter



Zif268 finger1 M E R P Y A C P V E S C D R R F S R S D E L T R H I R I H T G Q K

Zif268 finger2 P F Q C R I - - C M R N F S R S D H L T T H I R T H T G X X

random 30 resiude tail X X X X X X X X X X X X X X X X X X X X X X X X X X X X X X

Library size ~ 2.7 x 10^8

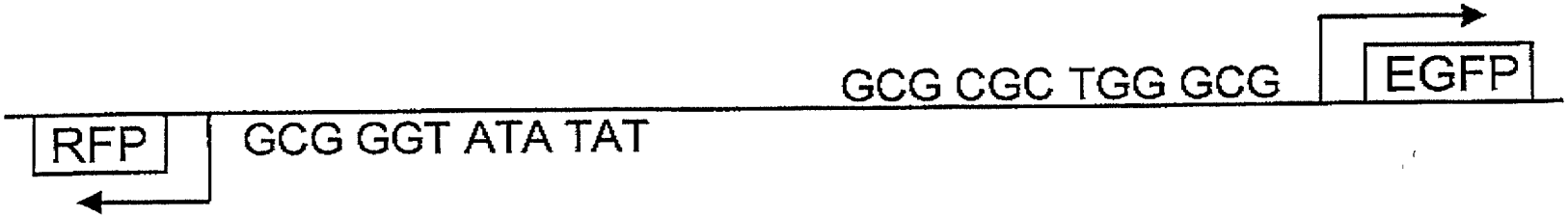


FIG. 15

Sequence of library and reporter

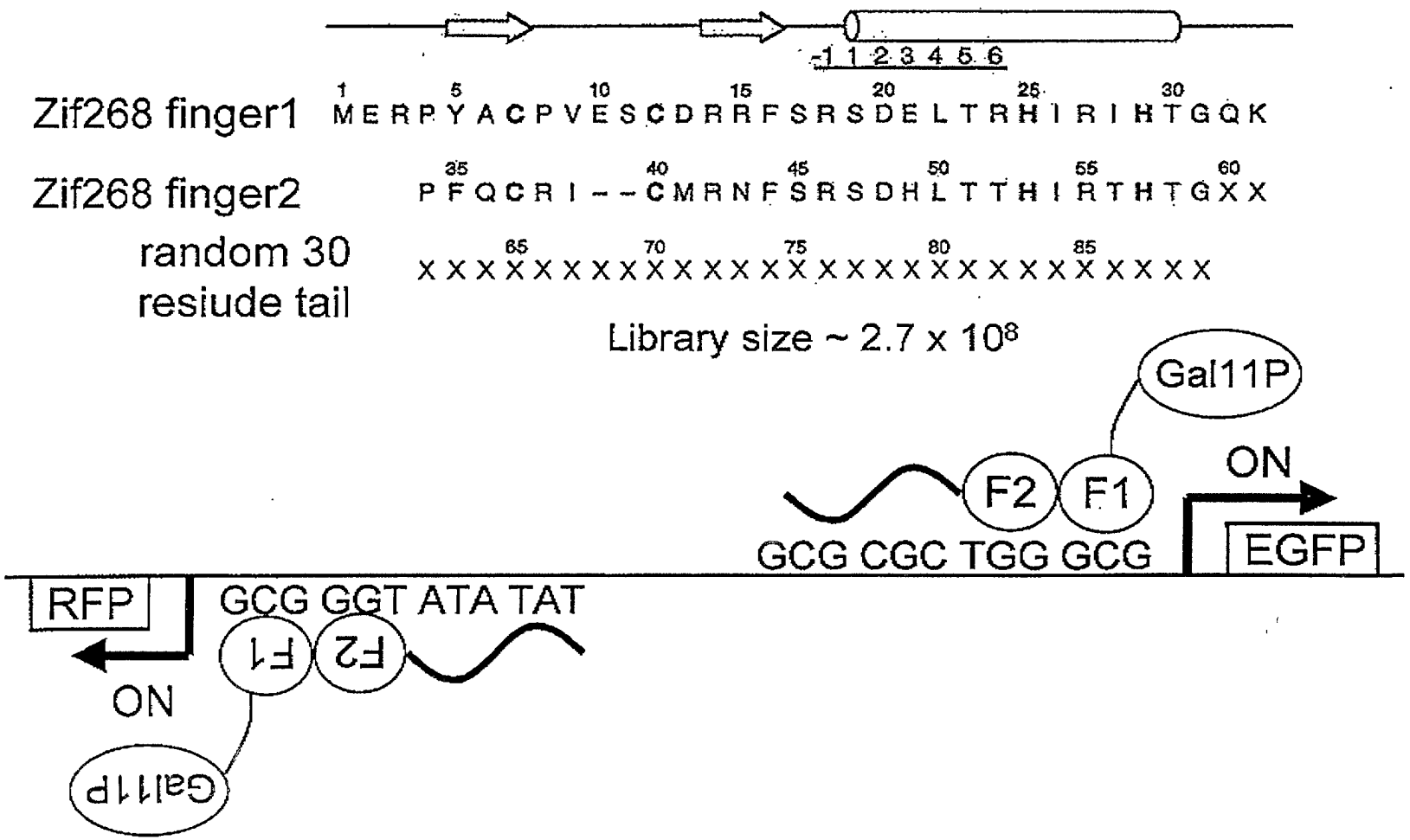
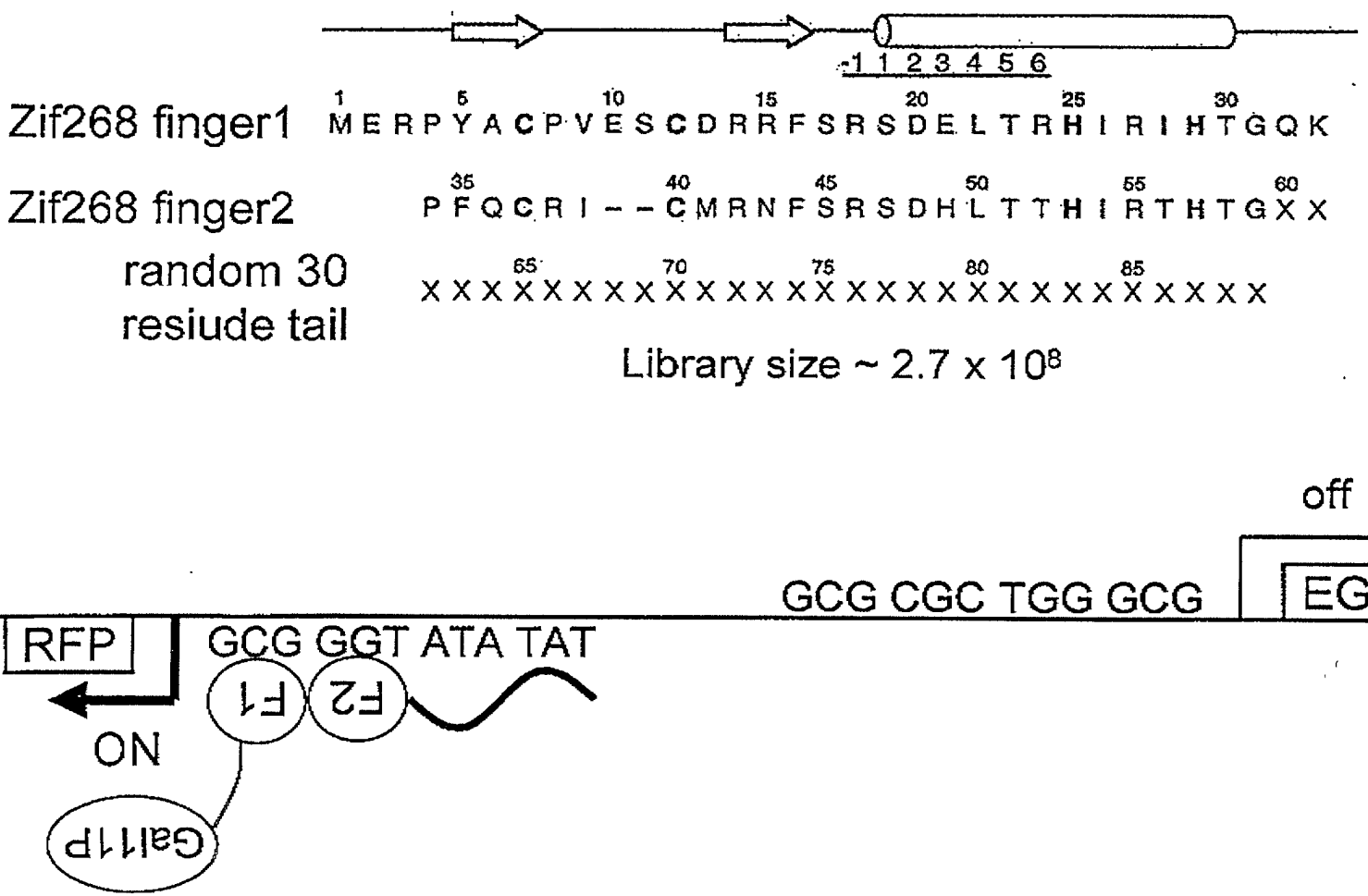


FIG. 16

Sequence of library and reporter



Sequence of library and reporter